

**WHAT IS CLAIMED IS:**

1. A method of screening for an antimicrobial agent, comprising the steps of:  
 (a) providing a test compound, a microbial proliferation gene and a first  
 and a second sample of a microorganism,

5 wherein the microbial proliferation gene is identified by introducing an  
 exogenous nucleic acid into the microorganism, the exogenous nucleic acid having  
 substantial sequence identity to an endogenous microbial gene, wherein the exogenous  
 nucleic acid is a random fragment or a random sequence, and, identifying the endogenous  
 gene as a microbial proliferation gene by comparing the proliferation or viability of the  
 10 microorganism when the exogenous nucleic acid is expressed in or introduced into the  
 microorganism with the proliferation or viability of the microorganism when the  
 exogenous nucleic acid is not present or not expressed,

(b) introducing the microbial proliferation gene into the microorganism of  
 the first sample;

15 (c) contacting the test compound with the first sample and the second  
 microorganism samples; and

(d) determining the effect of the test compound on the first and the second  
 microorganism samples, wherein the test compound is identified as an antimicrobial  
 agent by comparing the effect of contacting the test compound to the first sample, where  
 20 the exogenous nucleic acid is expressed or introduced, to the effect of contacting the test  
 compound to the second sample, where the exogenous nucleic acid is not present, and the  
 effect of the test compound on the contacted microorganism differs between the first and  
 the second samples, thereby identifying the test compound as an antimicrobial agent.

25 2. The method of claim 1, wherein the effect of contacting the test compound  
 with the microorganism is a change in the rate of proliferation of the contacted first  
 microorganism sample as compared to the contacted second microorganism sample,  
 thereby identifying the test compound as an anti-proliferative antimicrobial agent.

3. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a microbiostatic effect on the contacted first microorganism sample as compared to the contacted second microorganism sample, thereby identifying the test compound as a microbiostatic antimicrobial agent.

5

4. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a microbiocidal effect on the contacted first microorganism as compared to the contacted second microorganism sample, thereby identifying the test compound as a microbiocidal antimicrobial agent.

10

5. The method of claim 1, wherein the effect of contacting the test compound with the first microorganism sample as compared to the second microorganism sample is a change in the rate of transcription or amount of a transcription product of the microbial proliferation gene in the first microorganism sample.

15

6. The method of claim 5, wherein the transcription product is an RNA.

7. The method of claim 6, wherein the transcription product is an mRNA.

20

8. The method of claim 1, wherein the effect of contacting the antimicrobial agent with the microorganism is a change in the rate of translation or amount of a translation product of the microbial proliferation gene.

9. The method of claim 8, wherein the translation product is a polypeptide.

25

10. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a change in the activity of a translation product of the microbial proliferation gene.

30

11. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a change in metabolism of the contacted microorganism.

12. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a change in viability of the contacted microorganism.

13. The method of claim 1, wherein an effect of the test compound is  
5 determined indirectly.

14. The method of claim 13, wherein an effect of the test compound is determined by a protein activity assay.

10 15. The method of claim 14, wherein the effect of the test compound agent is determined by an enzyme assay.

16. The method of claim 13, wherein the effect of the test compound is  
15 determined by an immunoassay.

17. The method of claim 1, wherein the effect of contacting the test compound with the microorganism is a change in the rate of proliferation, metabolism or viability of the contacted first microorganism sample as compared to the contacted second microorganism sample, thereby identifying the test compound as an antibiotic.  
20

18. The method of claim 1, wherein introducing the microbial proliferation gene into the microorganism of the first sample results in an increased level of expression of the transcription or translation product of the gene, thereby requiring contacting more test compound to the first sample than to the second sample to have the same effect on  
25 the microorganism.

19. The method of claim 1, wherein introducing the microbial proliferation gene into the microorganism of the first sample results in a decreased level of expression or activity of the translation product of an endogenous microbial proliferation gene, thereby requiring contacting less test compound to the first sample than to the  
30 second sample to have the same effect on the microorganism.

20. The method of claim 19, wherein the microbial proliferation gene is operably linked to a transcriptional regulatory element effective for controlling expression of the exogenous nucleic acid.

5 21. The method of claim 20, wherein the transcriptional regulatory element effective for controlling expression of the microbial proliferation gene is an inducible regulatory sequence.

10 22. The method of claim 21, wherein the inducible regulatory sequence that controls expression of the microbial proliferation gene is a promoter induced in response to a chemical inducer.

15 23. The method of claim 22, wherein chemical inducer comprises isopropyl-beta-D-thiogalactopyranoside, tetracycline or tryptophan.

24. The method of claim 21, wherein the inducible regulatory sequence that controls expression of the microbial proliferation gene is a promoter induced in response to environmental changes.

20 25. The method of claim 20, wherein the microbial proliferation gene is operably linked in an antisense orientation to the transcriptional regulatory element.

25 26. The method of claim 20, wherein the microbial proliferation gene is operably linked in a sense orientation to the transcriptional regulatory element.

30 27. The method of claim 1, wherein introducing the microbial proliferation gene into the microorganism of the first sample results in an decreased level of expression of the transcription product of an endogenous microbial proliferation gene, thereby requiring contacting less test compound to the first sample than to the second sample to have the same effect on the microorganism.

28. The method of claim 27, wherein the microbial proliferation gene is operably linked to a transcriptional regulatory element effective for controlling expression of the exogenous nucleic acid.

5 29. The method of claim 28, wherein the transcriptional regulatory element effective for controlling expression of the microbial proliferation gene is an inducible regulatory sequence.

10 30. The method of claim 29, wherein the inducible regulatory sequence that controls expression of the microbial proliferation gene is a promoter induced in response to a chemical inducer.

15 31. The method of claim 30, wherein chemical inducer comprises isopropyl-beta-D-thiogalactopyranoside, tetracycline or tryptophan.

32. The method of claim 29, wherein the inducible regulatory sequence that controls expression of the microbial proliferation gene is a promoter induced in response to environmental changes.

20 33. The method of claim 28, wherein the microbial proliferation gene is operably linked in an antisense orientation to the transcriptional regulatory element.

25 34. The method of claim 28, wherein the microbial proliferation gene is operably linked in a sense orientation to the transcriptional regulatory element.

35. The method of claim 1, wherein the microbial proliferation gene is endogenous to the microorganism.

30 36. The method of claim 1, wherein the microbial proliferation gene is a bacterial proliferation gene.

37. The method of claim 36, wherein the bacterial proliferation gene has substantial sequence identity to the *E. coli viaA* gene.

38. The method of claim 36, wherein the bacterial proliferation gene has  
5 substantial sequence identity to the *E. coli orf1* gene.

39. The method of claim 36, wherein the bacterial proliferation gene has substantial sequence identity to the *E. coli lepB* gene.

10 40. The method of claim 36, wherein the bacterial gene has substantial sequence identity to the *E. coli ugpB* gene.

41. The method of claim 36, wherein the bacterial proliferation gene has substantial sequence identity to the *E. coli ddlB* gene.

15 42. The method of claim 36, wherein the bacterial proliferation gene has substantial sequence identity to the *E. coli secA* gene.

43. The method of claim 36, wherein the bacterial proliferation gene has  
20 substantial sequence identity to a gene having sequence identity to *E. coli fimF* or *fimD*.

44. The method of claim 1, wherein the microorganism is a pathogen.

45. The method of claim 1, wherein the microorganism is a bacterium.

25 46. The method of claim 45, wherein the bacterium is a pathogen.

47. The method of claim 45, wherein the bacterium is a gram-negative bacterium.

30

48. The method of claim 47, wherein the gram-negative bacterium is *Escherichia coli*.

49. The method of claim 47, wherein the bacterium is a gram-positive  
5 bacterium.

50. The method of claim 49, wherein the gram-positive bacterium is *Staphylococcus aureus*.

10 51. The method of claim 1, wherein the microorganism is a fungus.

52. The method of claim 1, wherein the microorganism is a yeast.

53. The method of claim 1, wherein the microorganism is an *Archaeobacteria*.  
15

54. The method of claim 44, wherein the microorganism is a human pathogen.

55. The method of claim 44, wherein the microorganism is an animal  
pathogen.  
20

56. The method of claim 44, wherein the microorganism is a plant pathogen.

57. The method of claim 1, wherein the exogenous nucleic acid is operably  
linked to a transcriptional regulatory element effective for controlling expression of the  
25 exogenous nucleic acid.

58. The method of claim 57, wherein the transcriptional regulatory element  
effective for controlling expression of the exogenous nucleic acid is an inducible  
regulatory sequence.

59. The method of claim 58, wherein the inducible regulatory sequence that controls expression of the exogenous nucleic acid is a promoter induced in response to a chemical inducer.

5 60. The method of claim 59, wherein chemical inducer comprises isopropyl-beta-D-thiogalactopyranoside, tetracycline or tryptophan.

61. The method of claim 58, wherein the inducible regulatory sequence that controls expression of the exogenous nucleic acid is a promoter induced in response to  
10 environmental changes.

62. The method of claim 57, wherein the exogenous nucleic acid is operably linked in an antisense orientation to the transcriptional regulatory element.

15 63. The method of claim 57, wherein the exogenous nucleic acid is operably linked in a sense orientation to the transcriptional regulatory element.

64. The method of claim 1, wherein the exogenous nucleic acid further comprises a vector.  
20

65. The method of claim 64, wherein the vector is a plasmid or a phage vector.

66. The method of claim 1, wherein the exogenous nucleic acid is from about 10 to about 5,000 nucleotides in length.  
25

67. The method of claim 66, wherein the exogenous nucleic acid is from about 15 to about 1,500 nucleotides in length.

68. The method of claim 1, wherein the exogenous nucleic acid is obtained  
30 from a nucleic acid selected from the group consisting of chromosomal DNA, episomal genomic DNA, RNA, cDNA and synthetic DNA.



69. The method of claim 1, wherein the test compound comprises a combinatorial library.

5 70. The method of claim 1, wherein the test compound comprises an inorganic compound.

71. The method of claim 1, wherein the test compound comprises an organic compound.

10 72. The method of claim 1, wherein the test compound comprises a peptidomimetic.

15 73. The method of claim 1, wherein the test compound comprises a polypeptide or a peptide.

74. The method of claim 1, wherein the test compound comprises an oligonucleotide or a polynucleotide.

20 75. The method of claim 1, wherein determining the effect of the test compound on the microorganism comprises comparative or replica plating of the microorganism.

25 76. The method of claim 1, wherein determining the effect of the test compound on the microorganism comprises measuring respiratory activity.

77. The method of claim 1, wherein determining the effect of the test compound on the microorganism comprises measuring colony-forming units, light scattering or optical density, the number of organisms in a particle counter, the fluorescence of cell cultures or of individual cells after addition of fluorescent dyes, the incorporation of precursors to macromolecules or the uptake of metabolites.

30

78. The method of claim 1, wherein the microorganism is a viable cell.

79. The method of claim 1, wherein the microorganism is a crude cell lysate.

5

80. The method of claim 1, wherein the test compound targets a transcription product of the microbial proliferation gene.

81. The method of claim 80, wherein the transcription product is an RNA.

10

82. The method of claim 81, wherein the transcription product is an mRNA.

83. The method of claim 80, wherein the test compound selectively binds to the transcription product.

15

84. The method of claim 1, wherein the test compound targets a translation product of the microbial proliferation gene.

85. The method of claim 84, wherein the translation product is a polypeptide.

20

86. The method of claim 84, wherein the test compound selectively binds to the translation product.

87. A method of screening for antibacterial agents, comprising the steps of:

25

(a) providing a test compound, a microbial proliferation gene and a first and a second sample of a bacterium,

wherein the bacterial proliferation gene is identified by introducing an exogenous nucleic acid into the bacterium, the exogenous nucleic acid having substantial sequence identity to an endogenous bacterial gene, wherein the exogenous nucleic acid is a random fragment or a random sequence, and, identifying the endogenous gene as a bacterial proliferation gene by comparing the proliferation or viability of the bacterium

30

when the exogenous nucleic acid is expressed in or introduced into the bacterium with the proliferation or viability of the bacterium when the exogenous nucleic acid is not present or not expressed,

(b) introducing the bacterial proliferation gene into the bacterium of the first sample;

(c) contacting the test compound with the first sample and the second bacterium samples; and

(d) determining the effect of the test compound on the first and the second bacterial samples, wherein the test compound is identified as an anti-proliferative anti-bacterial agent by comparing the effect of contacting the test compound to the first sample, where the exogenous nucleic acid is expressed or introduced, to the effect of contacting the test compound to the second sample, where the exogenous nucleic acid is not present, and the effect of the test compound on the contacted bacterium differs between the first and the second samples, thereby identifying the test compound as an anti-bacterial agent.

88. A cell system for identifying an antimicrobial agent, comprising a microbial proliferation gene, a first and a second sample of a microorganism, and instructions for

(a) identifying the microbial proliferation gene by introducing an exogenous nucleic acid into the microorganism, the exogenous nucleic acid having substantial sequence identity to an endogenous microbial gene, wherein the exogenous nucleic acid is a random fragment or a random sequence, and, identifying the endogenous gene as a microbial proliferation gene by comparing the proliferation or viability of the microorganism when the exogenous nucleic acid is expressed in or introduced into the microorganism with the proliferation or viability of the microorganism when the exogenous nucleic acid is not present or not expressed,

(b) introducing the microbial proliferation gene into the microorganism of the first sample;

(c) contacting a test compound with the first sample and the second microorganism samples; and

(d) determining the effect of the test compound on the first and the second microorganism samples, wherein the test compound is identified as an antimicrobial agent by comparing the effect of contacting the test compound to the first sample, where the exogenous nucleic acid is expressed or introduced, to the effect of contacting the test compound to the second sample, where the exogenous nucleic acid is not present, and the effect of the test compound on the contacted microorganism differs between the first and the second samples, thereby identifying the test compound as an antimicrobial agent.

10

15

20

25

30

10087895.doc